PIK3CA and AKT2 mutations of gastric cancer in China

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Abstract

Mutations in PI3K and/or AKT have been reported in a variety of cancers. This indicates that the two pathways interact to cause cancer. We have therefore investigated their roles in gastric cancer (GC) in China. In our study, exons 9, 18 and 20 of PIK3CA gene and exons 6~14 of AKT2 gene were screened in 10 GC cell lines and 100 advanced primary GC together with matched normal tissues. Denaturing high performance liquid chromatography (DHPLC) and DNA sequencing were used to analyze the mutations in the two genes. Two point mutations in the PIK3CA gene were identified in 4 of 10 GC cell lines and in 4 of 100 GC primary tumors. Two polymorphisms in AKT2 were detected in 19 of 100 GC primary tumors. One point mutation in AKT2 was detected in 1 of 10 GC cell lines and 3 of 100 GC primary tumors, and no hot spot variation was detected. Our results indicate that PIK3CA and AKT2 mutations are found in GC, although not a common event, therefore they might still play an important role in mediating kinase activities towards gastric carcinogenesis.

Keywords

PIK3CA; AKT2; gastric cancer; mutation

Introduction

Gastric cancer (GC) represents one of the most frequent malignant diseases in the world. However, the incidence and mortality of GC vary enormously in different countries. In China, the 5-year survival rate is below 20% and has remain unchanged in 20 years because most patients are presented at the advanced stage. Therefore, it is very critical to understand the mechanisms for GC which can be used for early diagnosis and improved therapy.

Mutations in PIK3CA and AKT2 have been reported in GC and in other cancers . The protein encoded by the PIK3CA gene represents the catalytic subunit, which powers phosphatidylinositol 3-kinase. Akt, also known as protein kinase B, is one of the major downstream effectors of PI3K . Upon activation, Akt moves to the cytoplasm and nucleus, and phosphorylates a number of downstream effectors to regulate various cellular functions. Therefore, perturbation of the PI3K/Akt pathway can cause the development of cancer.

In this study, we screened both tumor tissues and matched normal tissues, and GC cell lines for gene mutations by denaturing high performance liquid chromatography (DHPLC) and sequencing. Specifically, we addressed point mutation in hot spots of PIK3CA, and the polymorphisms and somatic mutations in the AKT2 kinase domain. With our systematic approach, we have identified the novel mutations of PIK3CA and AKT2 genes in GC, which might be important to GC carcinogenesis.

Materials and Methods

GC clinical specimens and GC cell lines

One hundred gastric carcinoma samples were collected in the Beijing Cancer Hospital, Beijing, China. All of the patients had undergone surgery with curative intent, and none of them had received preoperative adjuvant therapy. All tumors were histologically classified as stages III and IV according to the UICC TNM classification. Matched non-neoplastic normal samples were collected from the same gastric mucosa at around 5 cm away from the carcinoma tissues.

A panel of ten cell lines of GC was used as representing AGS, N87, MKN45, SNU-1, SNU-16, RF-1 obtained from American Type Culture Collection (ATCC), BGC823, MGC803, SGC7901 and PAMC82 established in China. The cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% FBS (Hyclone).

DNA isolation and PCR

Genomic DNA was extracted from frozen pathologic and normal tissue pairs through the standard phenol/chloroform and ethanol precipitation–extraction procedure. PCR amplification was performed in 25μL volume containing 100ng genomic DNA, 100μM each of dNTPs, 2mM $MgCl_2$, 2.5μL PCR buffer, 1μM each of forward and reverse primers and 1.5 units of Pfu DNA polymerase (Promega, US). Thirty cycles of PCR were performed with the following parameters: 95°C for 40s, 40s at an annealing temperature appropriate for each primer pair and 72°C for 80s, followed by 10min extension at 72°C. Oligonucleotide primers to amplify the segments of PIK3CA corresponding to exon 9, 18, 20 and AKT2 exon6 ~14 are listed in Table 1.

DHPLC analysis and DNA sequencing

Before analysis by DHPLC, all amplified fragments were heated to 95°C for 3 min followed by slow cooling to 45°C over 50 min to form a mixture of hetero and homoduplex molecules. The melting temperature and optimal gradient for each fragment can be obtained with WAVEMAKER 4.0 software with some empirical optimization. Aliquots of 5μL PCR products were automatically loaded on the DNASep column and eluted on a linear acetonitrile gradient in a 0.1M triethylamine acetate buffer (pH 7.0) with a constant flow rate of 0.9mL/min. Elution of DNA from the column was detected by absorbance at 260 nm. After detection of the abnormal elution peak in DHPLC analysis, the PCR products were purified by 4.0% polyacrylamide gels, and sequenced using forward/reverse primers with an ABI377 DNA automated sequencer (Perkin-Elmer, US). The sequencing to confirm the mutations were repeated three times.

Results and Discussion

We selected and screened 3 exons of the PIK3CA gene (exon 9, 18, 20), in which nucleotide variations were frequently reported in many kinds of tumors. We screened 110 gastric cancers (100 clinical specimens and 10 cell lines) for mutations of PIK3CA. Summaries of the PIK3CA mutational status are shown in Table 2 and Fig.1. A1634C transition was detected in gastric cancer cell line AGS and in one primary tumor. Such mutation is known to cause codon 545 to change from Glu→Ala. A2730G transition was detected in 3 gastric cancer cell lines and in 3 primary tumors, which are not known to cause amino acid changes. The PIK3CA mutation frequency in the 100 primary gastric cancers is 4.0%.

We selected and screened 9 exons of the AKT2 gene (exon 6 ~14), within which is the kinase domain. A polymorphic locus in codon 150 Asn→Ser of the AKT2 gene was detected with the wild type A/A frequency of 98% (98/100), and mutation type G/G frequency of 2% (2/100). A polymorphic locus in intron 48877 was also detected with the wild type A/A frequency of 83% (83/100), and homozygote type A/G frequency of 17% (17/100). A point mutation G→C in intron 43377 was detected in 1 gastric cell line and 3 primary tumor 3% (3/100) (Table 3, Fig.2).

Many cancers contain PIK3CA mutations and the majority of them are within exon 9, 18, and 20 . In our study, we focused to detect alteration in these same 3 exons. Three advanced GC tissue sample and 4 of 10 GC cell lines harbored the PIK3CA hot spot mutations. In addition, two point mutations were found in the AKT2 gene. The incidence of these PIK3CA and AKT2 mutations are at the low end of frequencies (2-11%) that were reported in the literature. Since these mutations are located in the kinase domain, an important functional area, our data confirm that these mutations contribute to the pathogenesis of gastric cancers, In addition, the similar mechanisms for induction of gastric cancer may exist in Chinese as in other populations.

There are two significant features regarding the PI3K and AKT genes. First, mutations in PI3K/AKT can cause extensive cellular disturbances. For example, proteins that crosstalk with PI3K/AKT signaling pathway include K-RAS, EGFR, ERBB2, and BRAF which are frequently mutated in human cancers. Second, protein kinase mutations, either gain or loss of function mutations, are important not only in cancer development, but also in cancer therapies. An impressive example of recent cancer therapies is the used of kinase inhibitors such as imatinib (Gleevec) and

gefitinib (Iressa) to target mutated kinases that play a dominant role in cancer progression . Thus, our study adds knowledge to the field of gastric carcinogenesis and therapy.

Table I

Primer sequences and PCR conditions for the AKT2 and PIK3CA genes

Gene	Exons	Primer	Squence(5′→3')	Size (bp)	Tm (℃)
AKT2					
	Exon6	F	AGC CGT TTG GCA ACA GTG TCT	350	60.0
		R	AAA TAA GCC CAC AGC AGC AGA		
	Exon7	F	GCC ACT GAT GAT CCT CGT CTG	324	58.5
		R	GGG CTC TCT CTC TGA GCT CTG		
	Exon8	F	AGC AGA GCC CTC CTC CCT CC	216	61.0
		R	CCT CCA CCC TTC CAT CTC AC		
	Exon9	F	GCC TTG ATT GGT CCC TCT AGC	340	61.5
		R	CCA ACT TCC CCA GTG TGA GT		
	Exon10	F	GGC ATG GGA GGG TTG ATG TC	320	59.6
		R	TGG GCT GGA AAC ACA CAG GT		
	Exon11	F	CAG CCC TCA TTT CTC CTC CA	355	61.2
		R	CGA CAC ACT GCG ACC CTA CA		
	Exon12-13	F	CTG CTC CGA AAG CCC GTC T	388	61.0
		R	CAG GCA CTC ACA GCG GTC AG		
	Exon14	F	GCC TTT CCT GTC CTG TCC TG	233	61.0
		R	CGA GCG TGC GTC CTC TGC G		
PIK3CA					
	Exon9	F	TTG CTT TTT CTG TAA ATC ATC TGT G	321	59.0
		R	CTG CTT TAT TTA TTC CAA TAG GTA TG		
	Exon18	F	TCC TTA TTC GTT GTC AGT GAT TG	348	54.0
		R	CAG CTT TCA AAA ATA AGA AAT TAT G		
	Exon20	F	GGA ATG CCA GAA CTA CAA TC	252	62.0
		R	TTT GCC TGC TGA GAG TTA TT		

Table II

Mutations of PIK3CA in GC cell lines and gastric tumors

Exon	Nucleotide	Gene type	Amino acid	Cell lines	Primary tumor
9	A1634C	A/C	E545A	1/10(10%)	0/100(0%)
18	A2730G	A/G	synonymous	3/10(30%)	3/100(3.0%)
20	No				

Table III

Mutations and polymorphisms of AKT2 in GC cell lines and gastric tumors

Exon/Intron	Nucleotide	Gene type	Amino acid	Cell lines	Primary tumors
Exon6	A652G	G/G	N150S	0/10(0%)	2/100(2%)
Intron5	G43377C	G/C		1/10(10%)	3/100(3%)
Intron9	A48877G	G/G		0/10(0%)	17/100(17%)

Figure 1

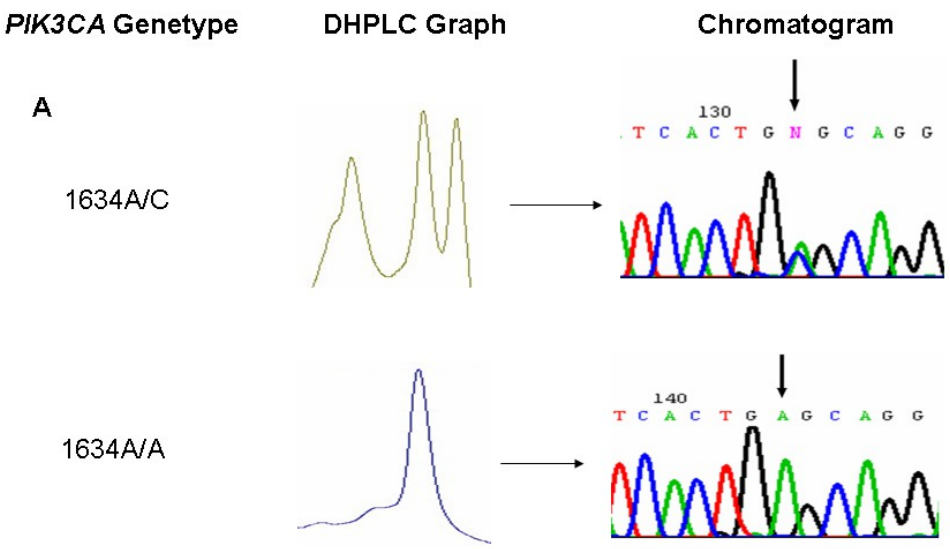

Figure 1

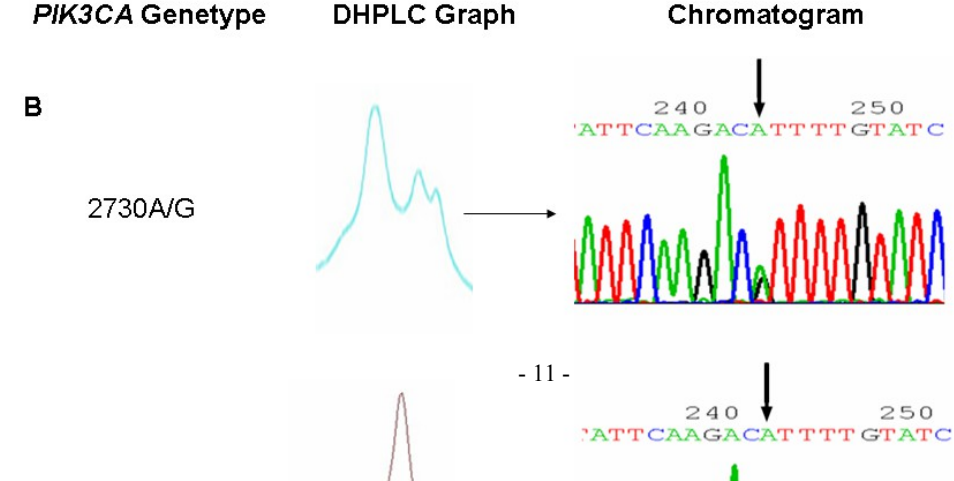

Figure 1. Genotype, DHPLC graph and chromatogram of *PIK3CA* gene. (A) Mutation in *PIK3CA* 1634 in exon9, single peak pattern of homozygote 1634 *A/A* genotype, three peak pattern of homozygote 1634 *A/C*. (B) Mutation in *PIK3CA* 2730 in exon18, single peak pattern of homozygote 2730 *A/A* genotype, three peak pattern of homozygote 2730 *A/G*.

Figure 2

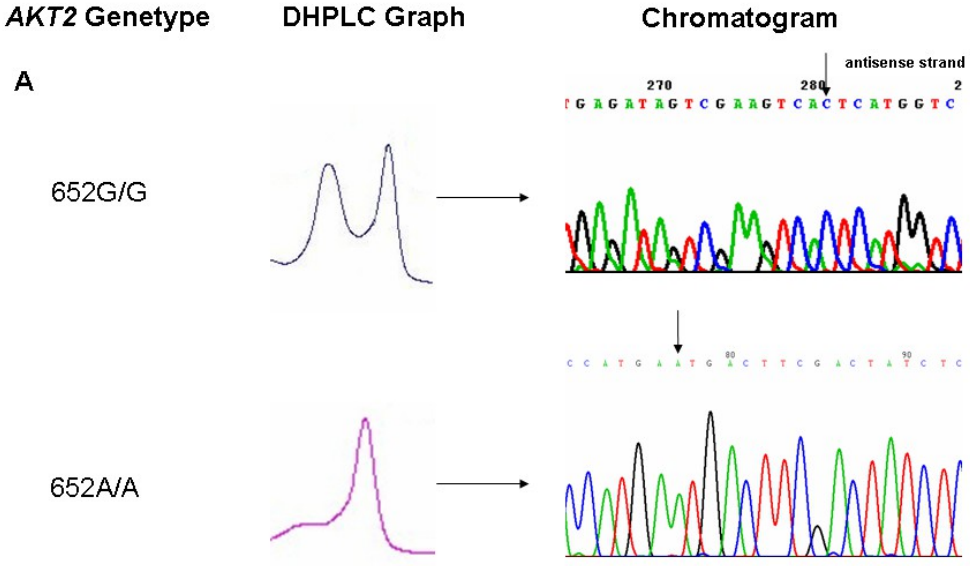

Figure 2

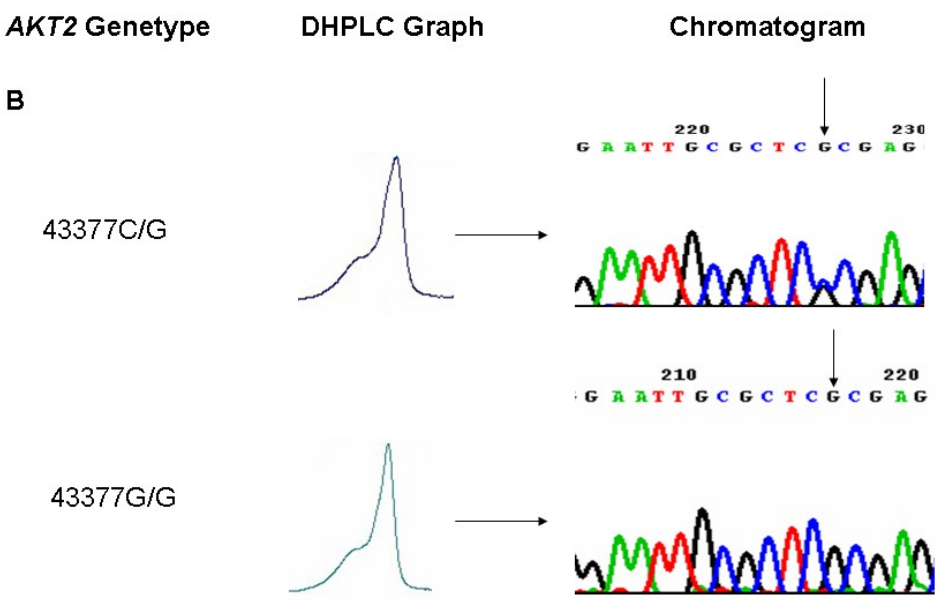

Figure 2

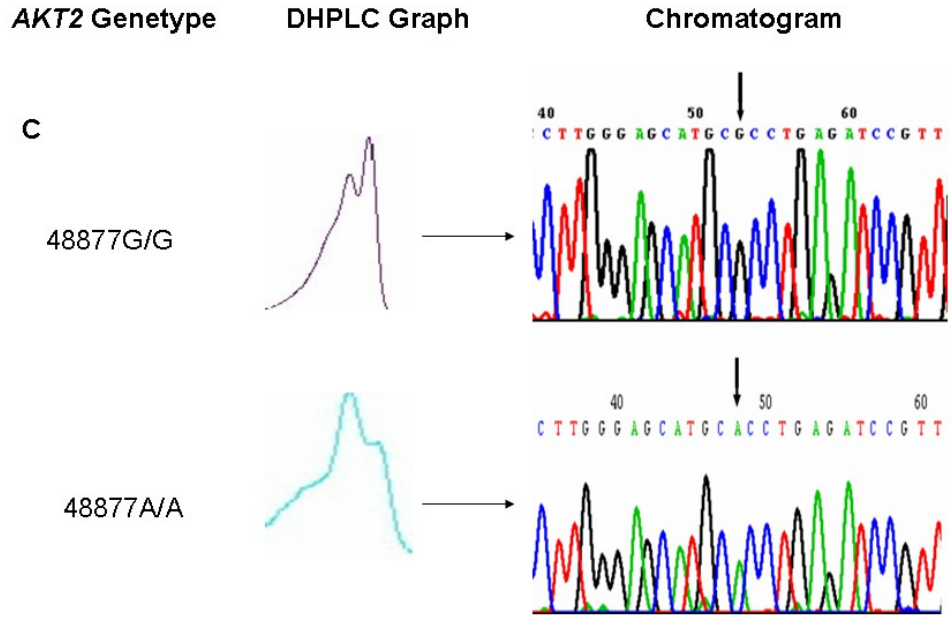

Figure 2. Genotype, DHPLC graph and chromatogram of *AKT2* gene. (A) Genotype of *AKT2* 1625 polymorphsim in exon6, single peak pattern of homozygote 652 *A/A* genotype, double peak pattern of homozygote 652 *G/G*. (B) Mutation in *AKT2* 43377 in intron6, single peak pattern of homozygote 43377 *A/A* genotype, double peak pattern of homozygote 43377 *C/G*. (C) Genotype of *AKT2* 48877 polymorphsim in intron6, double peak pattern of homozygote 48877 *A/A* genotype (first peak higher than second), double peak pattern of homozygote 48877 *G/G* genotype(first peak lower than second).

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Conflict of interest statement

We declare that we do not have a direct financial relation with the commercial identity mentioned in our paper. And we have no financial and personal relationships with other people or organizations that can inappropriately influence our work; there is no professional or other personal interest of any kind in any software, product, service and/or company that could be construed as influencing the position presented in the manuscript.

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